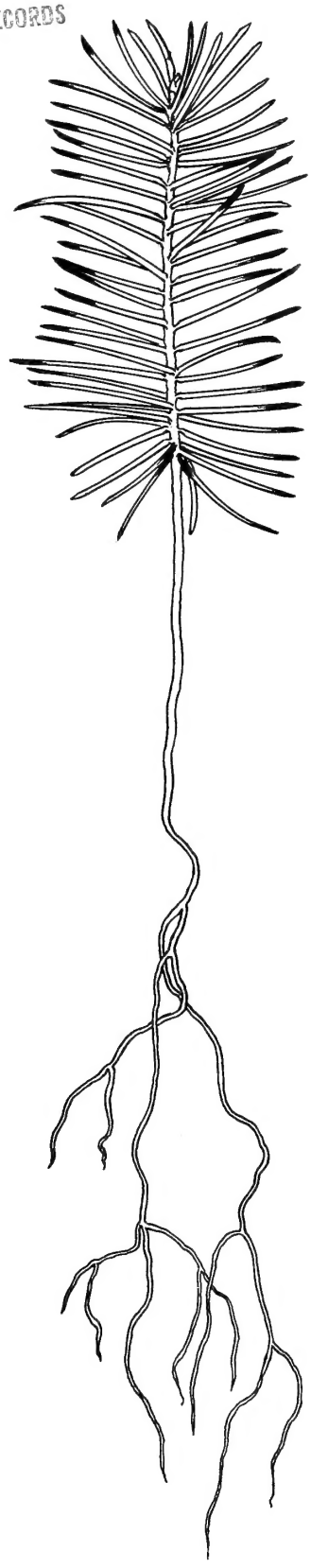
Absorption and Distribution of C^{14}-labeled TETRAMINE

in relation to its possible use in ANIMAL DAMAGE CONTROL



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INTRODUCTION

Tetramethylenedisulphotetramine (tetramine) is an extremely toxic chemical (Hagen 1950).[1] Shortly after its discovery in Germany (Hecht and Henecka 1949), it was introduced in the United States and found useful as an experimental seed protectant in reforestation by direct seeding (Spencer 1954).

The first indication of tetramine's systemic characteristics was that seedlings grown from tetramine-treated seed were toxic to rodents during the first month after germination (Spencer 1954). This observation led to (1) bioassay studies to determine translocation of the chemical and (2) pen and field tests to evaluate the effectiveness of tetramine as a systemic chemical for protecting seedlings against wild animals (Kverno 1960). In order to more fully evaluate the potential usefulness of tetramine for practical application, additional studies were undertaken of the chemical's translocation patterns in plants and its fate in plants and animals.[2] This paper, one of several reports on these studies, describes the patterns of absorption, translocation, and mobility of tetramine in three plant species.

[1] Names and dates in parentheses refer to Literature Cited, p. 16.

[2] All work with animals is in cooperation with the Olympia Field Station of the U. S. Bureau of Sport Fisheries and Wildlife, Olympia, Wash.

MATERIALS AND METHODS

Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco), blackberry (*Rubus ursinus* Cham. & Schlecht.), and orchard grass (*Dactylis glomerata* L.) served as experimental material. These plants, respectively, were used to represent gymnosperms, dicotyledonous angiosperms, and monocotlyedonous angiosperms. One seed lot of low-elevation Douglas-fir, one lot of orchard grass seed, and cuttings from one blackberry plant were used to grow plants needed for all experiments.

CULTURE OF PLANTS

Young plants of the three species were grown in sand until they reached a height of 3 to 5 inches. Plants were then washed free of sand, selected for uniform height, transferred to quart Mason jars covered with aluminum foil and containing Hoagland's nutrient solution 1 (Hoagland and Arnon 1950) with three parts per million (p.p.m.) of chelated iron, and placed in a plant growth chamber. Temperatures in the chamber were 26° C. during the day and 16° C. at night. Root zone temperature remained constant at 19° C. Relative humidity ranged from 70 to 90 percent, and light (at level of plant leaves) was controlled at 1,800 foot-candles on a 14-hour photoperiod. Culture solutions were continuously aerated, and plants were allowed to acclimatize in the chamber for at least 10 days before treatment.

TREATMENT OF PLANTS

C^{14}-labeled tetramine with a specific activity of 2.02 millicuries per millimole was prepared by Tracerlab, Waltham, Massachusetts. Stock solutions of the chemical were freshly prepared for the different experiments. Solutions for root and foliar treatments, respectively, were 20 p.p.m. in 1-percent acetone solution and 1,000 p.p.m. in 80-percent acetone solution containing 0.1-percent "Tween 20" surfactant.

Four tests were made with the tracer to determine (1) uptake by roots and distribution in the shoots, (2) mobility within treated plants, (3) distribution to flowering parts (blackberry only), and (4) movement following foliar applications.

UPTAKE BY ROOTS AND PROGRESSIVE DISTRIBUTION

Roots of grass, blackberry, and Douglas-fir plants were subjected to C^{14}-labeled tetramine (tetramine*) for 2, 6, 8, 24, and 96 hours. Tetramine* was used at the rate of 100 milliliters of stock solution (root treatment solution) to 700 milliliters of nutrient solution to form treatment solutions of 800 milliliters, each containing 2.0 milligrams tetramine* (17 microcuries). Four plants of each species, paired in two jars, were used per treatment. In each case, plants were transferred to treatment solutions and resulting cultures were moved immediately to the growth chamber. Growth conditions were the same as previously outlined and solutions were continuously aerated during the experiment. At the end of each exposure period in the time series, plants were removed from treatment solutions and processed as later described.

MOBILITY WITHIN PLANTS

Eight plants of each species were root treated with tetramine* as outlined above. Grass and blackberry were treated for 6 hours; Douglas-fir seedlings were treated for 48 hours. After treatment, roots were washed in distilled water and plants were transferred to tetramine-free nutrient solutions. Cultures were returned to the growth chamber and allowed to grow under the usual conditions. Solutions were changed every 2 days the first week and once a week thereafter. Four plants each of grass, blackberry, and Douglas-fir were harvested

after 7, 7, and 30 days, respectively. Remaining grass and blackberry were allowed to grow for an additional 7 days; Douglas-fir was grown for another 30 days before the experiment was terminated.

DISTRIBUTION IN FLOWERING PLANTS

Four blackberry plants with flower buds were treated for 48 hours with tetramine* via the roots as previously described. Roots were then washed in distilled water and the plants transferred to tetramine-free nutrient solutions. Cultures were returned immediately to the growth chamber, and solutions were changed every 2 days. Plants were harvested when most flowers had opened.

MOVEMENT AFTER FOLIAR APPLICATION

Four plants of each of the three species were used. Tetramine* was applied at the rate of 50 microliters of stock solution (foliar treatment solution) containing 50 micrograms tetramine* (0.42 microcurie) per plant. Solution was applied with a micropipette to one mature leaf of each grass and blackberry and to a group of mature needles of each Douglas-fir. Treatment was for 7 days under growth chamber conditions outlined above.

PROCESSING OF HARVESTED PLANTS

At time of harvest indicated for each experiment, roots of treated plants were washed thoroughly in distilled water and blotted with soft tissue. Treated leaves (from foliar application) were partly covered with masking tape to prevent contamination during further processing. Intact plants were then quick-frozen with crushed solid carbon dioxide and freeze-dried immediately at $-15°$ C. (Pallas and Crafts 1957).

ASSAY METHODS

The autoradiographic method (Yamaguchi and Crafts 1958) was used to determine distribution of C^{14} in treated plants. For each treatment, the best two plants were mounted on paper and placed against no-screen X-ray film for 4 weeks at room temperature. After exposure, films were developed by standard procedure to produce photographic images resulting from tracer radioactivity. The dark areas on resulting autoradiograms were used to determine location of the tracer in treated plants.

RESULTS AND DISCUSSION

Results of the different treatments are presented in the form of autoradiograms of representative plants.

Although the autoradiographic method has some limitations, in this study the method proved satisfactory. Control plants of the three species produced no pseudoautoradiograms, agreement among replications was good, and levels of activity employed were well within the limits of resolution of the X-ray film.

In presentation and discussion of results, C^{14} in the plants is, for simplicity, often referred to as tetramine*. Whether activity in the tissues was due to tetramine* or to a metabolite thereof is currently being investigated.

UPTAKE BY ROOTS AND PROGRESSIVE DISTRIBUTION

Autoradiograms and mounted plants of grass, blackberry, and Douglas-fir, representing progressive absorption and upward movement of tetramine* from root treatments of 2 to 96

hours, are shown in figures 1A to 1E. An examination of these autoradiograms shows that much higher concentrations of labeled material from nutrient solutions were retained in roots of the three species than were moved into the tops. This difference suggests that roots of the test species may fix, metabolize, or metabolically absorb tetramine*.

Differences between species in rates of translocation of the labeled material are also evident. In 2 hours (fig. 1A), Douglas-fir translocated almost no tracer to aerial parts; blackberry translocated enough so that the shoot image was very slightly visible; and grass moved sufficient material to the leaves to give a clearly visible autoradiogram of the whole plant. Only after 24 hours (fig. 1D) did activity become visible in tops of Douglas-fir. The slow movement in Douglas-fir was probably due to its characteristic meager root absorbing area, smaller area of transpiring foliage, and lack of vessels compared with the other species. These characteristics probably restricted uptake and transport in the xylem.

As absorption and translocation continued, the tracer accumulated in older parts of leaves where transpiration presumably occurred most rapidly. This pattern is clearly displayed in figures 1C and 1D in tips of grass leaves and in figure 3 at leaf margins of blackberry.

During the entire experiment, absorption of tetramine* continued, as evidenced by the sequence of autoradiograms in figure 1. Also, individual treatments showed presence of high activity in older leaves only, suggesting that the tracer was carried upward in the transpiration stream and retranslocation out of older leaves probably did not occur.

MOBILITY WITHIN PLANTS

Lack of mobility of tetramine* within plants is shown in figures 2 to 4. Initially (figs. 2A to 4A), slight movement into new growth (leaves without arrows in figure 2 and growth above the arrows in figures 3 and 4) occurred after removal of plants from treatment solutions. This movement probably was not derived from old growth (tops present at time of treatment). More likely, tracer moved into new growth from excess tracer in or on the washed roots.

As growth continued in tetramine-free solutions, old roots showed less tracer as they elongated and new roots contained little or no activity (figs. 2B to 4B). Similarly, little or no tracer was present in new aerial parts. In the meantime, old shoots and roots showed slight loss of activity, which was probably due to dilution by tissue growth or by degradation of tetramine*.

Tetramine*, therefore, was markedly immobile (nonsystemic) in the three species. Once deposited, it did not recirculate readily within the plant.

DISTRIBUTION IN FLOWERING PLANTS

Autoradiograms in figures 5A and 5B show appreciable activity in open flowers of blackberry plants which were treated after flowers had formed. The tracer was present in all flower parts, especially the calyx. Much less activity, however, appeared in the cluster of flower buds shown in figure 3B and marked by "X" where flowers were initiated after treatment and during growth in tetramine-free solution.

Apparently, tetramine* must be available to the roots during flowering for the tracer to appear in appreciable amounts in the flowers. Activity acquired before flowering is not readily available for redistribution and deposition in new flowers because of the chemical's marked immobility, as indicated in the mobility experiment above.

MOVEMENT AFTER FOLIAR APPLICATION

Areas of leaves and needles where the acetone solution was applied did not show any sign of damage to the tissues. The autoradiograms (fig. 6) show that movement out of treated leaves was, at best, very limited. Most noticeable movement occurred in grass (fig. 6A) where the tracer moved from point of application toward the tip of the treated leaf. Similar slight movement probably occurred in blackberry and Douglas-fir. Clear detection of the tracer on the autoradiograms (figs. 6B and 6C), however, was not possible because of shorter leaves of the species and the tape covering treatment areas. Figure 6 also shows no basipetal movement of tracer in any of the three species.

Movement of tracer toward tips of treated leaves in direction of the transpiration stream and not in direction of assimilate movement presents additional evidence (see above) of relative immobility of tetramine* in the plant. Applications of tetramine* to tops of plants, therefore, would not result in movement to roots.

SUMMARY AND CONCLUSIONS

Absorption, translocation, and mobility of C^{14}-labeled tetramine were studied in orchard grass, blackberry, and Douglas-fir plants. Tagged tetramine was applied to the roots in nutrient solutions and to the foliage, and the autoradiographic method was employed to determine distribution of the tracer.

Tetramine* was absorbed from nutrient solution by roots of the three species. Transport occurred via the transpiration stream and was apparently dependent on area of transpiring foliage and extent of absorbing root system. Thus, upward movement was slowest in Douglas-fir and fastest in orchard grass.

After uptake by roots and deposition in plant tissues, tetramine* did not recirculate within the plant in any of the species. Immobility of the chemical was also demonstrated by lack of downward movement with the assimilate stream after foliar applications to mature leaves. Tetramine, therefore, did not behave as a systemic chemical in the test species.

Appreciable amounts of tetramine* appeared in blackberry flowers when plants were treated after flower initiation had begun, but the chemical did not move to flowers formed after treatment had ceased.

In addition to tetramine's known toxicity, results point to serious limitations to its use to protect Douglas-fir seedlings from animals. If tetramine is applied to Douglas-fir in the nursery before outplanting, protection would last through the first dormant season only. New growth would not be protected because of the chemical's immobility (nonsystemic properties). In this case, field applications would be necessary to protect new growth. On the other hand, if tetramine is applied in the field, the chemical becomes available to other plant species associating with Douglas-fir (e.g., grass and blackberry). Foliage and fruit of these plants may become as toxic — or perhaps more so — as the trees, presenting an additional hazard to man, livestock, and wild animals unless tetramine is metabolized into a safer compound.

FIGURE 1A. TREATMENT PERIOD: 2 HOURS

Comparative uptake and upward movement of tetramine-C^{14} in grass, blackberry, and Douglas-fir through a 96-hour period. Tetramine was administered via the culture solution at 2.5 p.p.m. for several treatment periods. Autoradiograms appear above the mounted plants.

FIGURE 1B. TREATMENT PERIOD: 6 HOURS

FIGURE 1C. TREATMENT PERIOD: 8 HOURS

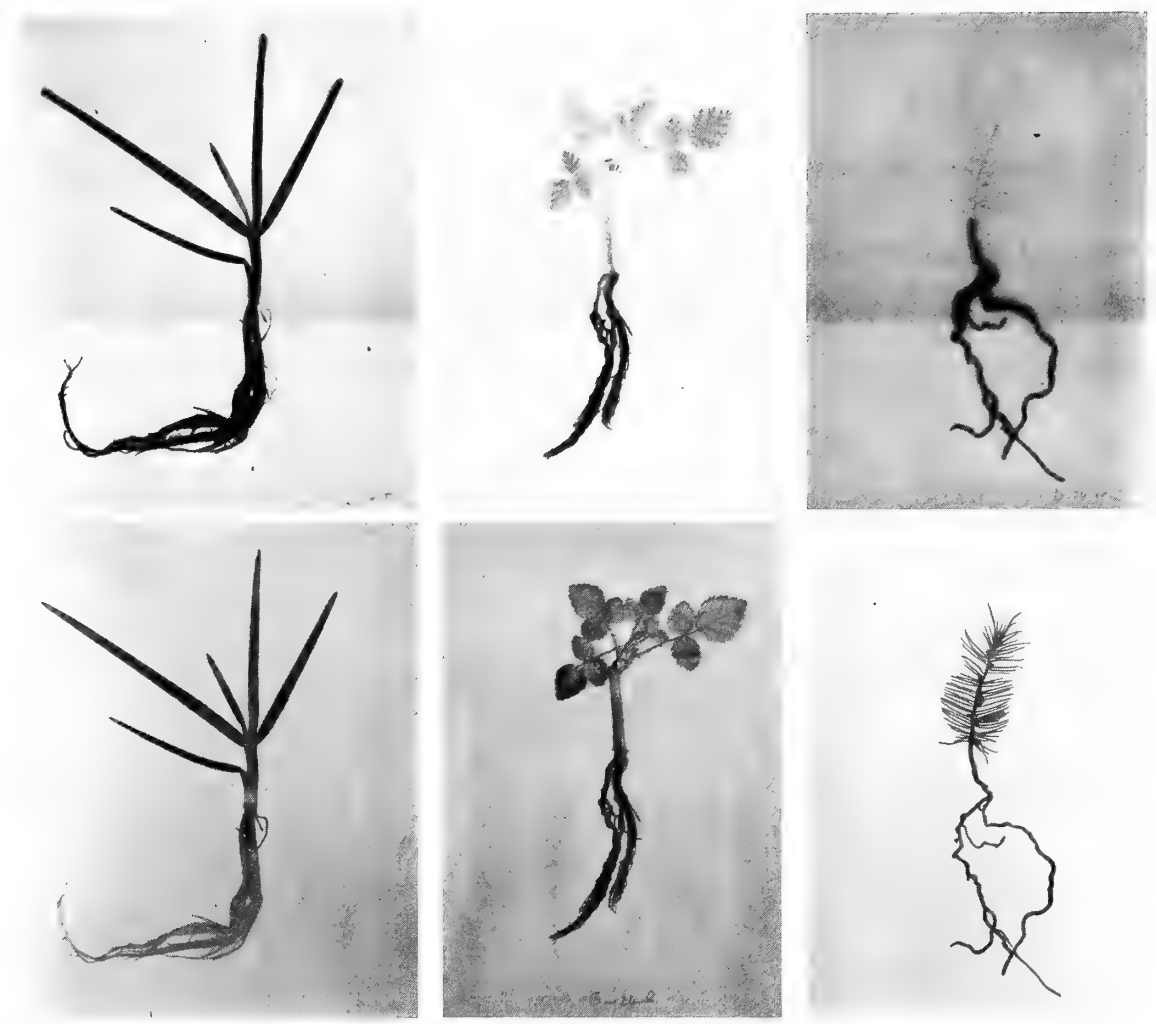
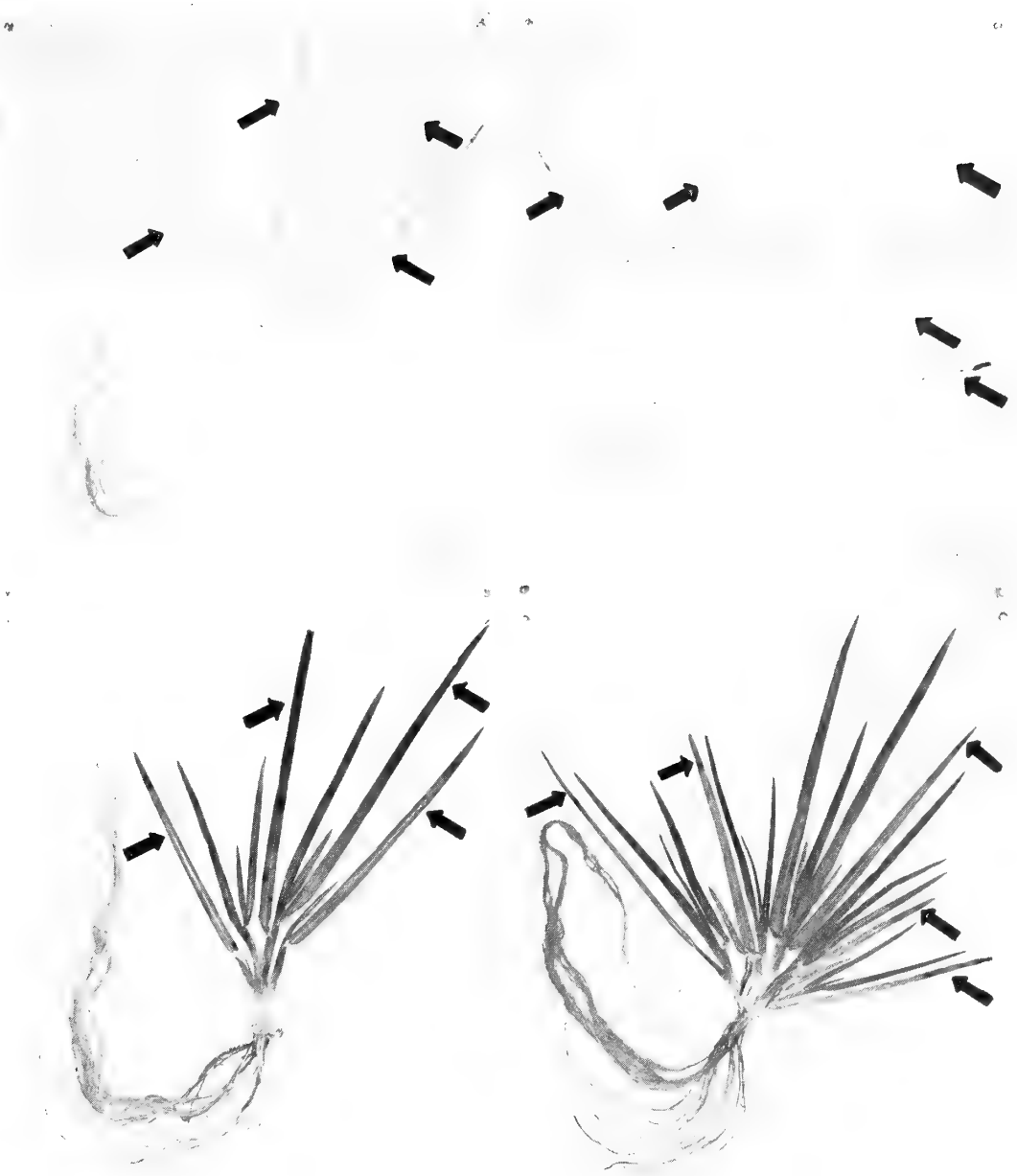


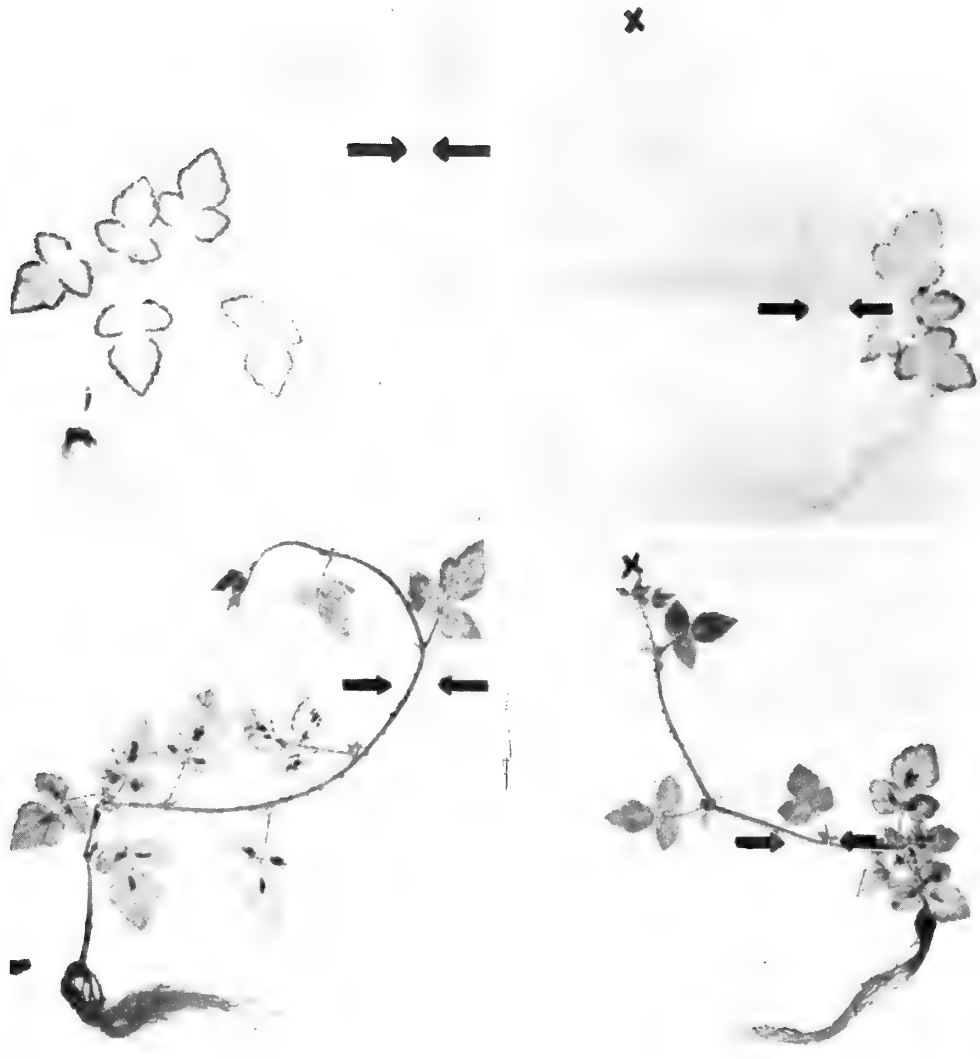
FIGURE 1D. TREATMENT PERIOD: 24 HOURS

FIGURE 1E. TREATMENT PERIOD: 96 HOURS



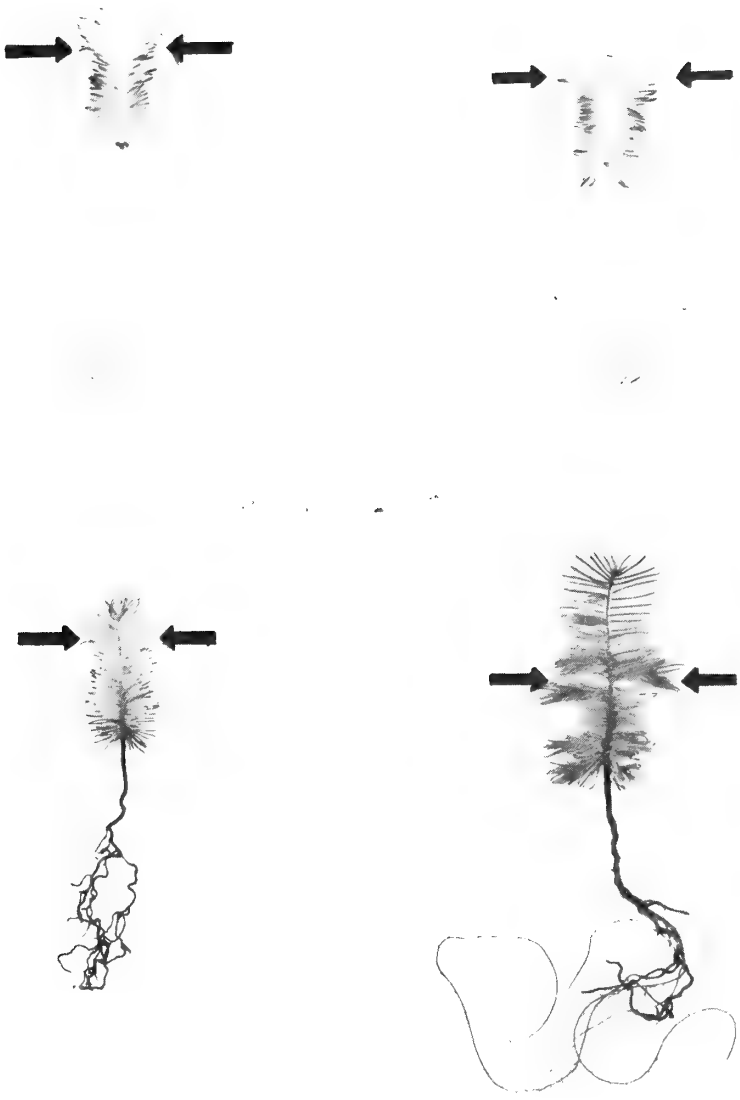
A. 7 DAYS' GROWTH B. 14 DAYS' GROWTH

FIGURE 2. Redistribution of tetramine-C^{14} in grass. Seedlings were treated for 6 hours with tetramine-C^{14} in culture solution at 2.5 p.p.m. before they were transferred to tetramine-free solution and allowed to grow. Upper section shows autoradiograms of treated seedlings shown in lower section, and arrows point to leaves present at time of treatment.



A. 7 DAYS' GROWTH — B. 14 DAYS' GROWTH

FIGURE 3. Redistribution of tetramine-C^{14} in blackberry. A and B treatments are as in figure 2, and autoradiograms are shown above treated plants. Arrows point to position of apical bud at time of treatment and X indicates flower buds in B.



A. 2-DAY TREATMENT
30 DAYS' GROWTH IN
TETRAMINE-FREE SOLUTION

B. 2-DAY TREATMENT
60 DAYS' GROWTH IN
TETRAMINE-FREE SOLUTION

FIGURE 4. Redistribution of tetramine-C^{14} in Douglas-fir. A and B are as in figure 2, except for treatments of 2 days and post-treatments in tetramine-free solutions of 30 days (A) and 60 days (B). Autoradiograms are shown above treated plants, and arrows point to position of apical bud at time of treatment.

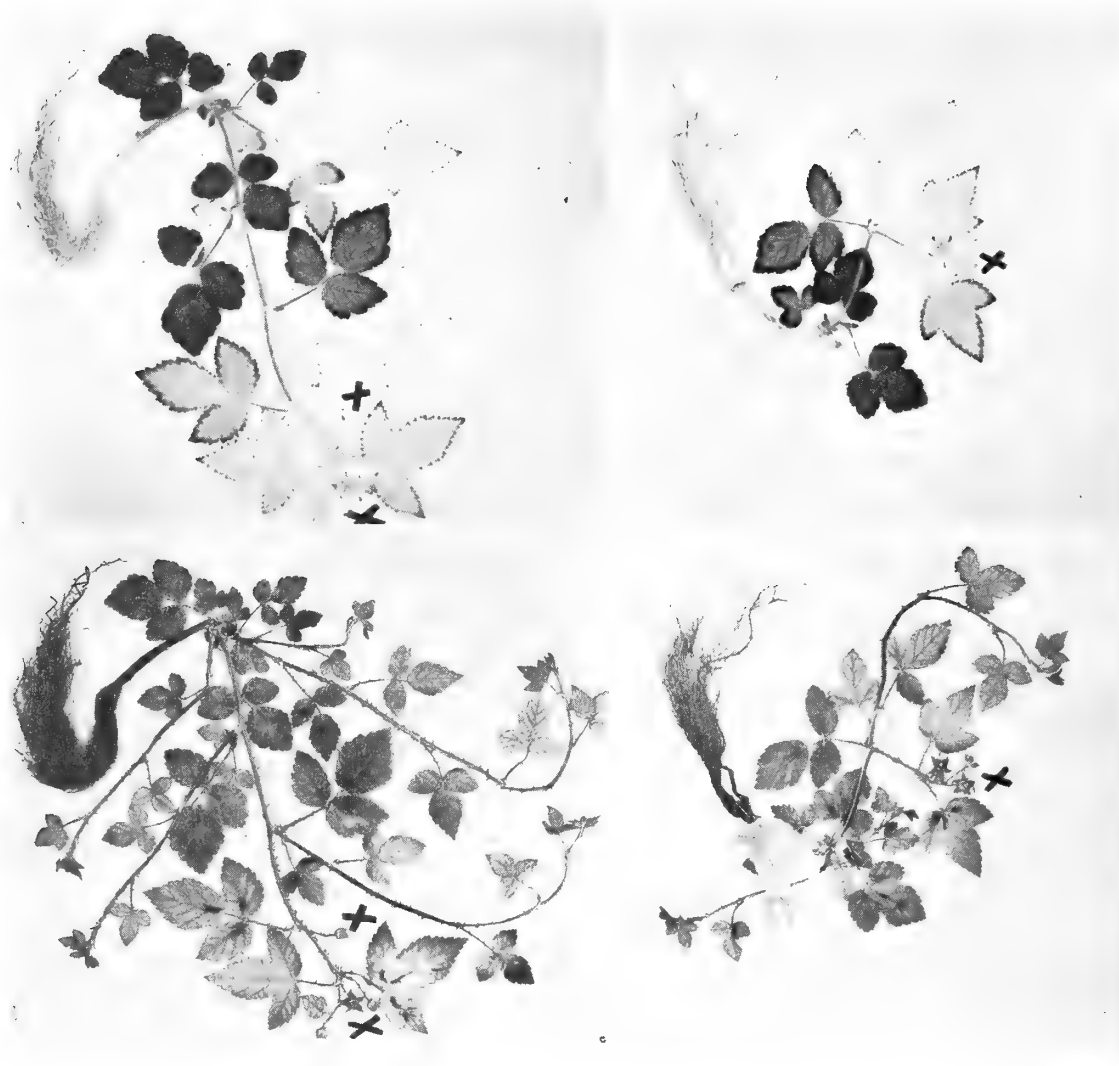


FIGURE 5. Distribution of tetramine-C^{14} in flowering blackberry. Plants with flower buds were treated with tetramine-C^{14} for 48 hours as in figure 2, and then allowed to grow in tetramine-free solution until flowers were fully developed. Autoradiograms are shown above and treated plants below; X indicates position of flowers.

LEAVES OF GRASS BLACKBERRY DOUGLAS-FIR

FIGURE 6. Comparative movement of tetramine-C^{14} from leaves of grass, blackberry, and Douglas-fir. Leaves indicated by arrows were treated for 7 days with 50 micrograms tetramine-C^{14} in 50 microliters of acetone solution. Upper section shows autoradiograms of treated plants shown in lower section.

LITERATURE CITED

HAGEN, JOSEF.

1950. Schwere Vergiftungen in einer Polstermobelfabrik durch einen neuartigen hochtoxischen Giftstoff (Tetramethylendisulfotetramin). Deut. med. Wochensch. 75: 183-184.

HECHT, G., and HENECKA, H.

1949. Über ein hochtoxisches Kondensationsprodukt von Sulfamid und Formaldehyd. Angew. Chem. 61: 365-366.

HOAGLAND, D. R., and ARNON, D. I.

1950. The water-culture method for growing plants without soil. Calif. Agr. Exp. Sta. Circ. 347, 32 pp., illus.

KVERNO, NELSON B.

1960. The problems in the use of systemic rodenticides. Soc. Amer. Forest. Proc. 1959: 97-98.

PALLAS, J. E., JR., and CRAFTS, A. S.

1957. Critical preparation of plant material for autoradiography. Science 125: 192-193.

SPENCER, DONALD A.

1954. Rodents and direct seeding. J. Forest. 52: 824-826.

YAMAGUCHI, S., and CRAFTS, A. S.

1958. Autoradiographic method for studying absorption and translocation of herbicides using C^{14}-labeled compounds. Hilgardia 28: 161-191.

Radwan, M. A.

1966. Absorption and distribution of C^{14}-labeled tetramine in relation to its possible use in animal damage control. U. S. Forest Serv. Res. Pap. PNW-34, 16 pp., illus. Pacific Northwest Forest & Range Exp. Sta., Portland, Oreg.

Absorption, translocation, and mobility of radioactive tetramine were studied in three plant species. Tetramine was absorbed by roots of all plants; upward movement, however, was slowest in Douglas-fir, intermediate in blackberry, and fastest in orchard grass. After absorption by roots, tetramine was immobile (nonsystemic) within the plants. Nonsystemic properties were also indicated by lack of downward movement following foliar applications. Results suggest that use of tetramine to protect forest tree seedlings from animals does not look promising.

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